

# GERANIUM STEMROT CAUSED BY *PYTHIUM COM- PLECTENS* N. SP.

## HOST RESISTANCE REACTIONS; SIGNIFICANCE OF *PYTHIUM* TYPE OF SPORANGIAL GERMINATION<sup>1</sup>

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### INTRODUCTION

Geranium cuttings (*Pelargonium*) are often affected with a blackening and decay of the roots and stems, which may result in complete rotting of the young plant. During the latter part of 1919, *Pythium de baryanum* and three other fungi belonging to the same genus were isolated from blackened geranium stems in the agricultural greenhouses at Washington, D. C., and were found capable of reproducing this condition in healthy cuttings. This paper is an account of one of these isolations, which differed very markedly in morphological and cultural characters from *P. de baryanum*, previously reported by Peters (14)<sup>2</sup> and recently by Buddin and Wakefield (5) as causing a geranium stemrot. It was further frequently characterized by the stimulation of a definite resistance reaction on the part of the host, evidenced by the formation of a cork cambium within and across the stem at some point in advance of infection, barring further progress of the hyphae after infection and rotting had already proceeded some distance from the point of inoculation.

### SIGNS OF THE DISEASE

The early stage of the disease, as caused by any of the *Pythium* spp. isolated, consists essentially of a progressive blackening and necrosis accompanied by wet rot of noncutinized and nonlignified tissues, usually commencing at the base of the cutting below the ground. Infected plants are not firmly embedded in the soil and offer little resistance to pulling, owing to their inability to form binding secondary roots or destruction of these when already present. General turgid-

ity is not affected at first, so that diseased cuttings may appear normal to the eye until the discoloration has progressed above ground.

After this stage the signs caused by the organism at present under consideration may be distinguished from those caused by the other *Pythium* spp. isolated, by the deeper dead-black discoloration, which progresses much more slowly up the stem and finally stops, usually 20 to 40 mm. above ground. This results in a sharp line of demarcation between the healthy, turgid green tissue and the shriveled black diseased portion below (Pl. 1, A). Under very warm, moist conditions, or when soil nematodes are abundantly present in the rotted tissues, infection may continue until it involves the entire plant, which wilts and rots on the ground. The characteristic limitation of infection is, however, more frequently observed in both naturally infected and artificially inoculated plants, often within six days after inoculation. Cuttings showing this type of infection may remain turgid above the dead portion of the stem so long as four weeks. No further growth takes place, however, and the plants remain dwarfed but turgid until they finally topple over through some external mechanical cause or through the continual weakening of the supporting base. If the stoppage of infection occurs sufficiently near the surface of the ground, secondary roots may be put forth just above the line of demarcation.

A more detailed examination of a diseased cutting in the early stage of infection shows a narrow gray to brown advancing margin, involving all stem tissues. Below this the discoloration is a deeper brown to black, and is accompanied by loss of turgidity in pith and cortex. These tissues are crushed in

<sup>1</sup> Received for publication April 16, 1924—issued January, 1925.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 419.

through the release of centrifugal pressure on the epidermal layers, and are rapidly hollowed out by a soft, wet rot. The cutinized epidermis, the underlying cork layers when present, and the lignified elements of the fibrovascular system are not included in this decay but form a hollow double cylinder, the outer (epidermal) firm and continuous, the inner (vascular) shredded and fibrous. Rotting of cortex and pith finally stops at the point of demarcation. Leaf scars included in the diseased area are hollowed out, leaving holes in the epidermal cylinder, through which a black exudate from the decaying tissues may be squeezed out.

Microscopic examination reveals hyaline, nonseptate hyphæ ramifying within and between the cells of the discolored tissues. Oospores with the peculiar antheridia characteristic of this organism are found within the infected cells just before their turgidity is destroyed, and are particularly abundant in the crushed cells of the later stage. Bacteria and nematodes are often present in later stages in the decaying tissue, but not in the turgid advancing brown area in which the coenocytic hyphæ may be observed reaching into the healthy cells beyond.

#### ISOLATION OF THE CASUAL ORGANISM

The causal organism was first isolated in November, 1919, from two out of six blackened geranium stems collected in the agricultural greenhouses. The remainder yielded *Pythium de baryanum*, which had been isolated earlier in the work and appears to be the pathogen most frequently associated with the stemrot. The fungus under discussion has since been repeatedly obtained and recognized in plantings from naturally infected cuttings from Washington and from collections made at Enid, Okla. (1923).

Stems from which isolations were to be made were first scrubbed free of adhering soil, then rinsed in alcohol and immediately flamed. They were then bisected longitudinally downward, beginning at the healthy portion above the advancing margin. A preliminary split was made with a sterile scalpel and the two halves were pulled apart with sterile forceps, thus ex-

posing infected internal tissue untouched by any outer agent. Bits of tissue at the infection margin were quickly excised with a flamed scalpel and planted on corn-meal agar plates. Isolations were also made by searing the surface of the cleaned stem with a hot knife and digging underneath with a flamed scalpel or needle. The first method was usually found more satisfactory, since one could see and select transplant material sufficiently remote from the badly decayed interior to minimize contamination by secondary organisms.

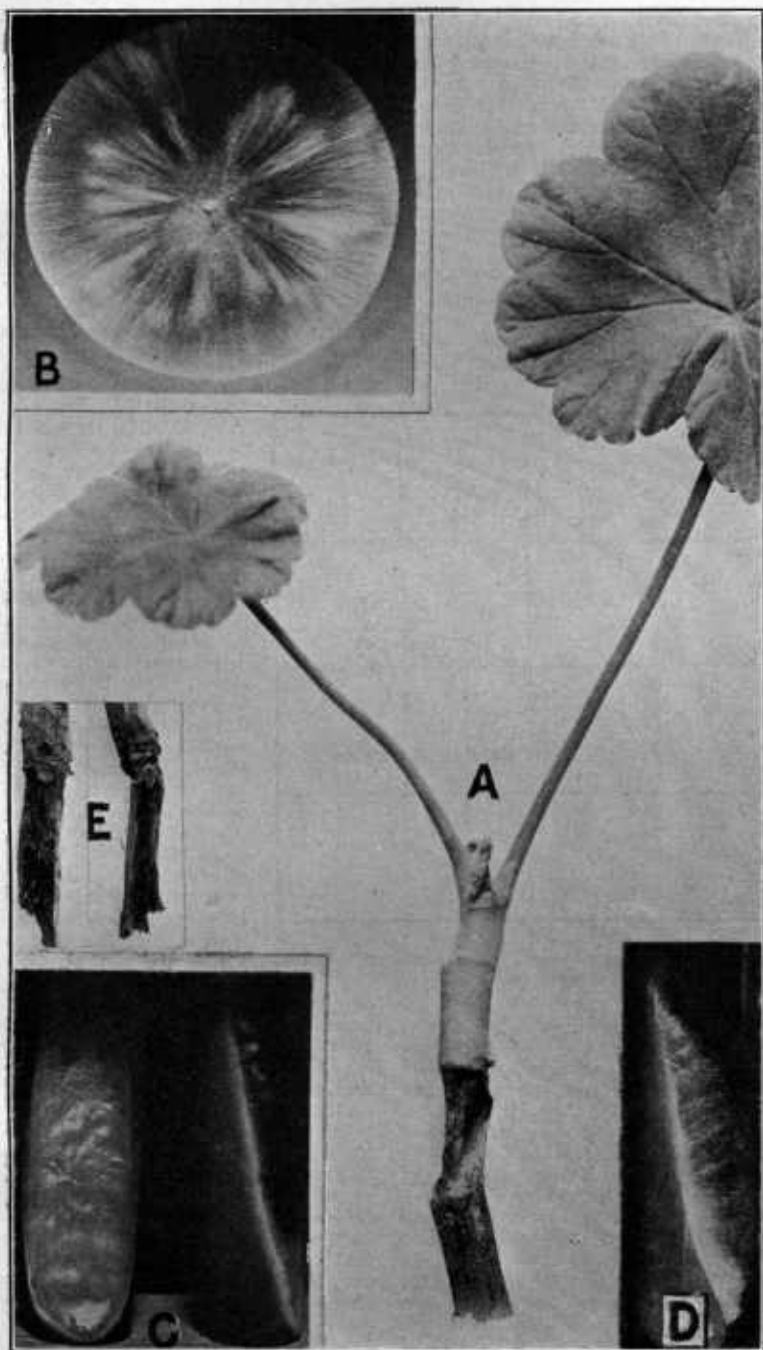
Isolations on corn-meal agar from slightly discolored advancing areas usually yielded colonies which for this organism were characterized by a compact prostrate growth of closely parallel silky hyphæ, later forming an abundance of oospores immediately around the transplant material. The fungus was obtained with difficulty from stems which were hollowed out up to the demarcation; the plates were often overrun with bacteria, nematodes, and soil fungi. In the absence of other fungi, the *Pythium* could be freed from bacteria by planting part of the growth in the center of another corn-meal agar plate, to which a drop of 50 per cent lactic acid had been added. Transfers were finally made to oatmeal agar tubes for use as stock cultures, after purity had been assured by the absence of bacterial growth in transfers on beef agar plates.

#### INFECTION EXPERIMENTS

Inoculations have been repeated at various intervals during the past four years, and have shown that the typical blackening and decay can be produced in healthy geranium cuttings when a portion of a pure culture is placed in contact with any wounded part of the stem. Mycelium from oatmeal agar or corn-meal agar cultures was generally used. Inoculations without previous injury were not successful. Since natural infections appeared to progress from the base, it was concluded that the freshly cut base of the stem placed in the soil was the usual means of entry of the organism. Cuttings placed in sterilized soil and inoculated at the base showed within 24 hours a sinking in of the pith and a

#### EXPLANATORY LEGEND FOR PLATE 1

- A.—Geranium cutting eight days after artificial inoculation. Note demarcation at node, shriveled black area below, turgid healthy tissues above
- B.—Colony on corn-meal agar plate, natural size, 48 hours at 25° C.
- C.—Carrot agar cultures one week old. Front and side views
- D.—Potato dextrose agar culture one week old. Side view
- E.—Decayed base, advanced state; hollowed out, epidermis and F. V. B. remaining



Geranium Stemrot

(For explanatory legend see p. 400)

brown discoloration and soft rot which spread upward with decreasing rapidity, finally stopping within six to eight days at some point 20 to 40 mm. up the stem. Below this point the decay continued until the epidermal cylinder and the fibrovascular system alone were left. Oospores could be found in infected tissues within four days after inoculation. Reisolation of the organism and successful inoculations with the reisolation completed the pathogenicity cycle. In checks where the severed surface was not inoculated a callus and roots soon formed, binding the plant into the soil, in sharp contrast with the loose condition of inoculated plants.

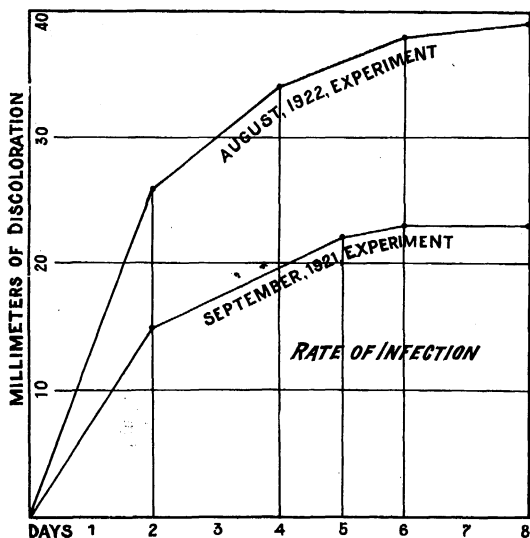


FIG. 1.—Graph showing progress of discoloration of inoculated geranium cuttings, 2 experiments, averages of 9 and 10 plants, respectively

In one experiment in which cuttings were placed in pots which had previously contained artificially infected plants, two out of five developed the stemrot, and the typical oospores were found in the tissues, indicating the rôle of infected soil as the carrier of inoculum. The large number of oospores usually formed and set free by decay of the host cells is doubtless a fruitful source of infection. Since their germination has not been observed, however, even after three months, it seems more likely that the primary source of infection lies in the mycelium and sporangia in decayed tissues, although the latter are not formed so abundantly as the oospores.

Cross-inoculations on cucumber, water cress, and radish seedlings in pots and in sterile tube cultures were un-

successful, except that superficial black streaks appeared on the radish stems without progressing much farther or causing any wilting. Infection was obtained on *Coleus* cuttings, which were rapidly discolored and subjected to a shriveling dry rot without any stoppage of infection as in the case of geranium cuttings.

Measurements of the progress of infection in the latter were made in two experiments, including 9 and 10 infected plants, respectively, and are plotted in Figure 1.

#### SUMMARY OF INOCULATION EXPERIMENTS

DEC. 3, 1919.—Twelve cuttings inoculated at the surface of the ground, six of these without wounding; five checks. All the plants were covered with bell jars. No infection after three weeks in checks and uninjured cuttings, callus and roots forming normally; discoloration and decay followed wound inoculations, rotting entire base but stopping above ground within six to eight days. Reisolutions made December 8 yielded 11 similar colonies of slow-growing type with combed-silk effect, like original. Typical oospores appeared on all the colonies.

JAN. 6, 1920.—Six cuttings inoculated at surface of ground, with slight scalpel injury; 10 inoculated at freshly cut base; six checks. Spreading discoloration visible the next day, except in two inoculated at surface, in which the inoculated area dried up and remained uninfected. By the eighth day decay had progressed up the stem from basal infections and had stopped with sharp demarcation. Lateral infections spread in both directions, destroying tissues below. The checks were healthy and formed roots.

FEB. 16, 1920.—Four cuttings inoculated with one of the December 8 reisolutions. All blackened on fifth day 8 to 22 mm. up to the stem; no further progress after ninth day; oospores abundant in the tissues. One check. This remained healthy.

MAR. 3, 1920.—Ten cuttings inoculated at base with original isolation. Eight infected; blackening progressed 11 to 20 mm. by fifth day. Further advance very slow, stopping at eighth day. Two inoculated plants and four checks remained healthy.

MAR. 20, 1920.—Five fresh cuttings planted in pots previously containing soil and debris of infected plants of March 3; no direct inoculation made from cultures. Two cuttings planted in fresh pots as checks. Two of the former showed typical infection on sixth day; oospores present. Remainder healthy.

SEPT. 15, 1921.—Ten cuttings inoculated at base. Nine infected. Progress of discoloration shown in Figure 1. The three checks remained healthy.

AUG. 17, 1922.—Ten cuttings, basal inoculation; soil sterilized three times. Sunken base within two days; for discoloration progress see Figure 1. Sharp demarcation eighth day; pith and cortex rotted. Typical colonies obtained in reisolutions made August 22. Five checks. These remained healthy.

JULY 26, 1923.—Thirty inoculated. Ten plants (set A) were placed on moist sand for two days before inoculation. The remainder (set B) were inoculated immediately on removal from stock plants. All examined a month later, August 26. Seven of set A healthy. Four of set B completely rotted down; remainder showed sharp demarcation, still alive and turgid, over hollow blackened stem below; epidermal cylinder intact, fibrovascular bundles forming a shredded inner cylinder; one plant had three adventitious roots starting from above the demarcation, which took place just above ground. Diseased areas 20 to 45 mm. long. Abundant typical oospores in tissues. The 10 checks remained sound.

SEPT. 24, 1923.—Three *Coleus* cuttings inoculated. The inoculated plants rotted completely within five days, without demarcation. The one check remained healthy.

OCT. 18, 1923.—Seedlings of water cress, cucumber, and radish in duplicate 6-inch pots inoculated with and without scalpel injury. No infection in any after two weeks.

FEB. 2, 1924.—Seedlings of cucumber and radish in glass moist chamber were inoculated, without injury. There was no infection on cucumbers. Black streaks appeared at points of inoculation on radish stems without progressing much or affecting turgidity.

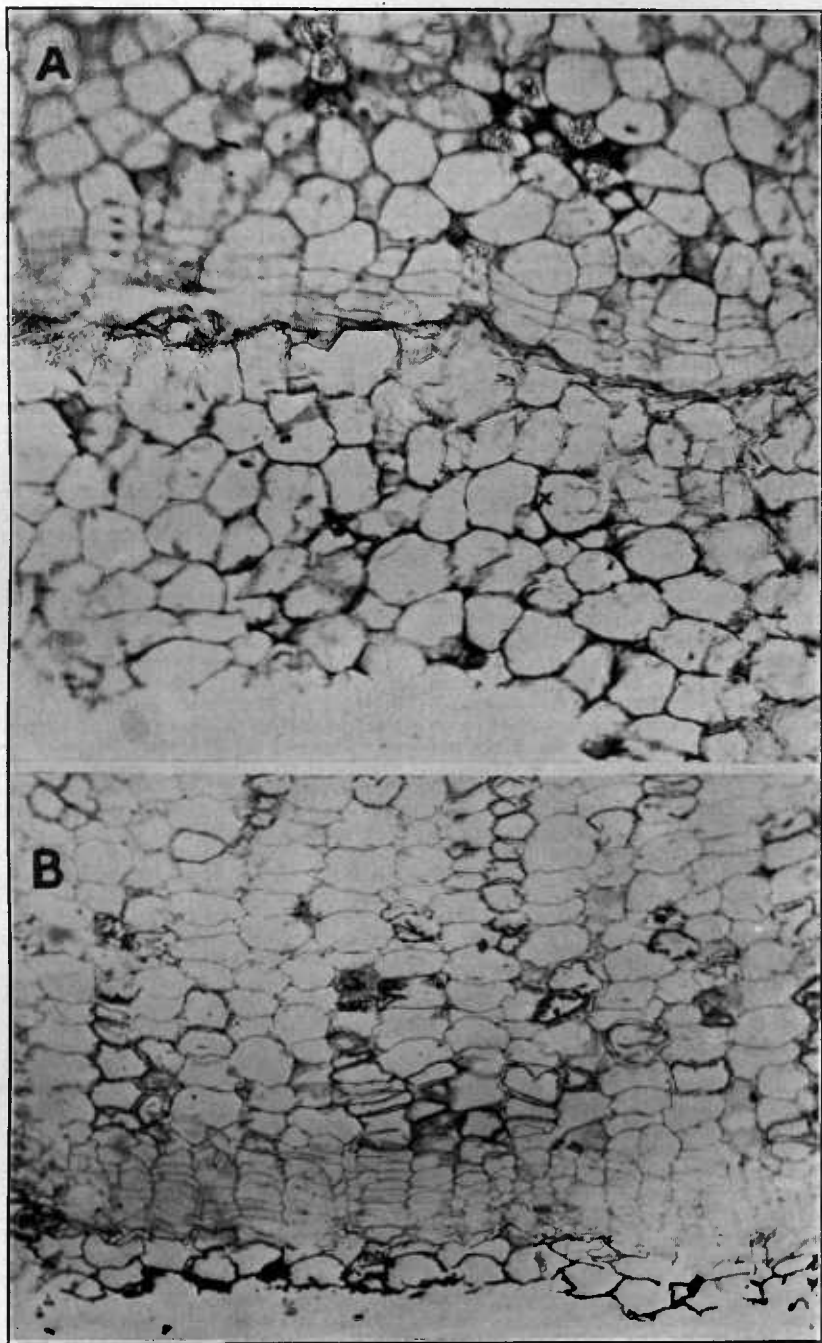
FEB. 16, 1924.—Seedlings of cucumber and radish (six each) grown under sterile conditions on Knopf's agar in test tubes, inoculated with and without wounding. The mycelium made some growth on the agar, but the cucumbers remained turgid and were not discolored. Black streaks not present in the checks appeared on radish stems but without affecting turgidity.

## PATHOLOGICAL HISTOLOGY

The hyphae are largely intracellular and show a constriction when passing through cell walls. They are found one or two cell layers ahead of cells with discolored walls, indicating that there is no lethal action in advance or that penetration is more rapid than visible degeneration and discoloration. Branched coils and nests of hyphae may be observed within the older infection area. Long strands run within and between sieve tubes and companion cells, with branches reaching out into cortex and pith. Oospores are present in great abundance within cells some distance back of the advancing margin and may be observed in progressive stages of maturation in a single section. Xylem and the cuticularized epidermis are not invaded. Penetration of the subepidermal cork layers from within rarely proceeds farther than the innermost, youngest, and least suberized layer.

Infected cells are killed immediately or very shortly after penetration, the walls soon taking up the brown discoloration. The thin-walled pith cells are the first to lose turgidity, resulting in the sunken appearance of the pith at the base of infected cuttings. Later the cortex also breaks down. The region of collapsed cells is often sharply delimited from adjoining infected but still turgid tissues (Pl. 2). Both pith and cortex are soon hollowed out by gelatinization and solution of their walls. The host nucleus is discolored but not corroded, and may be recognized for a long time after the cell has collapsed. Starch appears to be as abundant in diseased as in healthy tissues, except as noted below; mature oospores in collapsed cells may often be found among masses of uncorroded starch grains still embedded in their plastids.

Stained sections through the stem at the line of demarcation reveal an interesting condition. A cork cambium 4 to 12 cells thick extends irregularly but completely across, marking off healthy from diseased tissues (Pl. 3). It is usually indented upwards when crossing the fibrovascular bundles, along each side of which it may extend for some distance. The walls of the lowermost cambium layers (nearest the diseased tissues) are collapsed and take the gentian violet in the Flemming triple stain, indicating suberization. This conclusion has been confirmed by microchemical tests with Sudan III, Scarlet Red, alcoholic solution of chlorophyll, iodine and sulphuric acid, alkannin, and iodine. The walls of



Geranium Stemrot

(For explanatory legend see p. 406)

the more turgid upper phellogen cells, as well as the healthy parenchyma above, are deeply stained with the Orange G, indicating their cellulose nature. Starch has completely disappeared for a considerable distance above and below the cork layer. Immediately underneath the collapsed stratum of suberized walls are one to four layers of infected cells, whose walls are still rigid, slightly swollen, often split and pulled apart, and characterized by failure to take up completely any of the stains in the Flemming triple or the iodine green-acid fuchsin combination after fixation with Merkel's or Flemming's Strong. They remain a clear amber color, as in sections of fresh material. Negative reactions were obtained for lignin, suberin, pectin, and cellulose, except that solution took place in concentrated sulphuric acid. The contents are disorganized, and hyphæ (often oospores) are present, reaching up to the suberized layer but not beyond it.

Below this "nonstaining" layer is the remainder of the infected tissue, bordering on the hollowed part of the stem. The walls are swollen and stain deeply with the safranin of the Flemming triple, indicating pectinization. This was confirmed by positive reactions with Bismark Brown, Methylene Blue, and Ruthenium Red. Examination of walls in the "nonstaining" region bordering on the pectinized cells showed in many cases an extension of the safranin along the outer lamina. Thus, a single cell often showed complete reddening of the basal wall immediately adjacent to the pectinized tissues, the remaining walls being amber colored except for a short continuation of safranin on the outer parts of the two walls neighboring on the wholly stained wall. The fact that safranin is taken up only on the surface of the "nonstaining" wall implies action of pectinase from without, and precludes the possibility that it is due to incomplete washing out during the staining process, which would have left the safranin in the innermost lamella. That complete pectinization and solution up to the suberized layer ultimately takes place was shown by examination of cuttings which had been infected for several weeks and had formed the usual demarcation line. Here complete hollowing out had taken

place and the suberized layers, now several cells thick, bordered directly on the cavity (Pl. 3).

The formation of a cork layer across the stem acts as an effective barrier to further progress of the hyphæ, which have not been observed above it except where fissures have occurred through the action of nematodes or mechanical injury. It is clearly a specific reaction on the part of this host to this organism, since it was not present in the case of the other three *Pythium* species studied nor in the case of *Coleus* cuttings infected with this fungus.

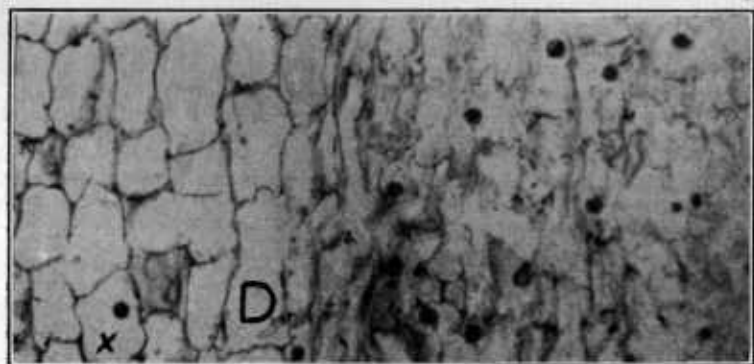
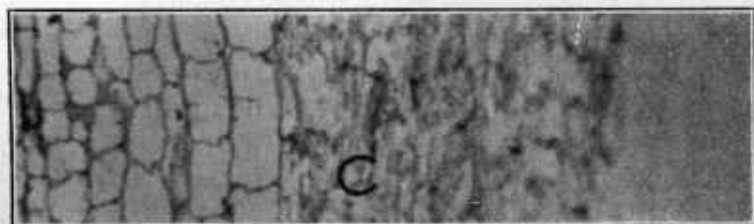
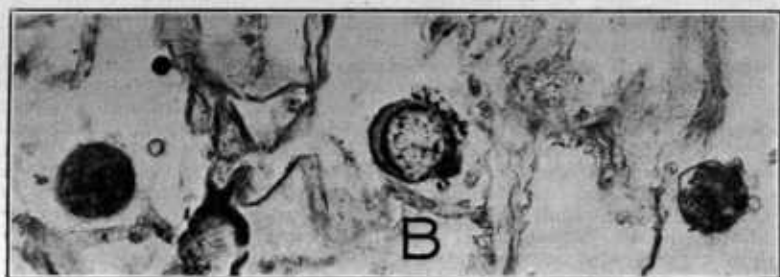
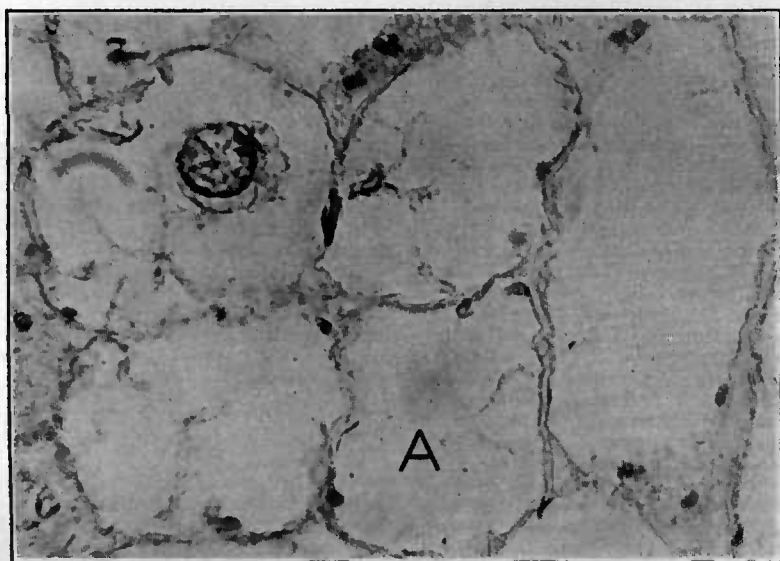
#### MORPHOLOGY AND DEVELOPMENT OF THE FUNGUS

**THE HYPHÆ.**—Hyaline, coenocytic when young, cylindrical, with no tapering in main or lateral branches (Pl. 4, E). They are very slender, measuring  $1.70\ \mu$  to  $4.85\ \mu$  (75 measurements). Branching is abundant and irregular; lateral hyphæ often extend beyond the parent hypha and become main branches in turn. The angle of branching varies from  $45^\circ$  to  $90^\circ$ , followed by a sharp swing forward, which results in a characteristic closely parallel growth on solid media, like combed silk. Smaller subsidiary branches are given off profusely and curve irregularly. A transparent Liesegang-ring effect in the otherwise dense growth has been found to be caused by a comparative scarcity of these smaller branches in these rings, thus affecting the otherwise uniform density of the growth. When growing plate cultures are inverted under the microscope, the hyphæ at the edge of the colony sometimes cease elongation at their original diameter, and put forth a slender prolongation from the tip, which broadens out farther on into the normal diameter and resumes the usual course of growth.

**SPORANGIA.**—The asexual fruiting bodies are always borne singly. They are usually terminal, less frequently intercalary (pl. 4, F), and sometimes sessile, owing to continued lateral growth of the hyphal tip from the base of the sporangium. Terminal and sessile sporangia are uniformly oval to spherical. Intercalary sporangia are irregularly oval to subspherical, often asymmetrical, with flattenings at the places of attachment. Measurements

#### EXPLANATORY LEGEND FOR PLATE 2

- A.—Section of stem at demarcation. Healthy cells at top; cork cambium center; below, single layer of crushed cells, suberized; split and swollen walls of infected cells below, deeply stained; hyphæ (at 2); pectinization of lower cell walls, causing cavity  
B.—Later stage, cambium layers increased; decay of diseased tissues has progressed up to the protective cork cambium





of 100 sporangia from corn-meal agar plates average  $21.85\ \mu$ , with ranges from  $16.4\ \mu$  to  $27.26\ \mu$ . Carrot agar and corn-meal agar are most favorable for their formation. They appear within four days after inoculation, on the upper surface of the agar, rarely within the agar or at the interface of agar and glass.

The walls are smooth, thin, and hyaline. No papilla has been observed. The contents are hyaline and finely, uniformly granular, with a varying number of small, round, darker spots which are evidently nuclei, judging from comparison with stained sections. Newly formed sporangia are nonvacuolate; older sporangia contain an irregular central vacuole, which increases with age, and occupies one-fifth to one-half the diameter of the sporangium.

The formation of the sporangia is readily observed in inverted blocks of corn-meal agar or in drops of oatmeal decoction, and presents the usual features found in *Pythium*—the swelling up of a hyphal tip (or segment when intercalary) into a globular body, which is then cut off by a septum, with occasional continued growth of the hypha laterally in the case of sessile sporangia. They are not readily detached, and often germinate *in situ*.

For about 10 days after their formation, the asexual fruiting bodies germinate by the extrusion of the undifferentiated contents through a short straight tube into an evanescent, delicately walled vesicle, where the zoospores are differentiated and whence they escape into the surrounding water. As older sporangia are placed in conditions favoring germination, the percentage germinating in this manner decreases, and a greater number protrude a tube, which continues to grow, branches, and forms a mycelium. Both of these methods of germination have been watched repeatedly and are described below in detail (Pls. 4 and 5).

A surface scraping near the point of inoculation of a 6-day-old corn-meal agar plate culture usually yields an abundance of sporangia, which may be placed for observation in a hanging drop of distilled water, previously aerated by a vigorous shaking of the bottle. Within 15 minutes a broad, thin-walled tube is seen protruding from most of the sporangia in the drop.

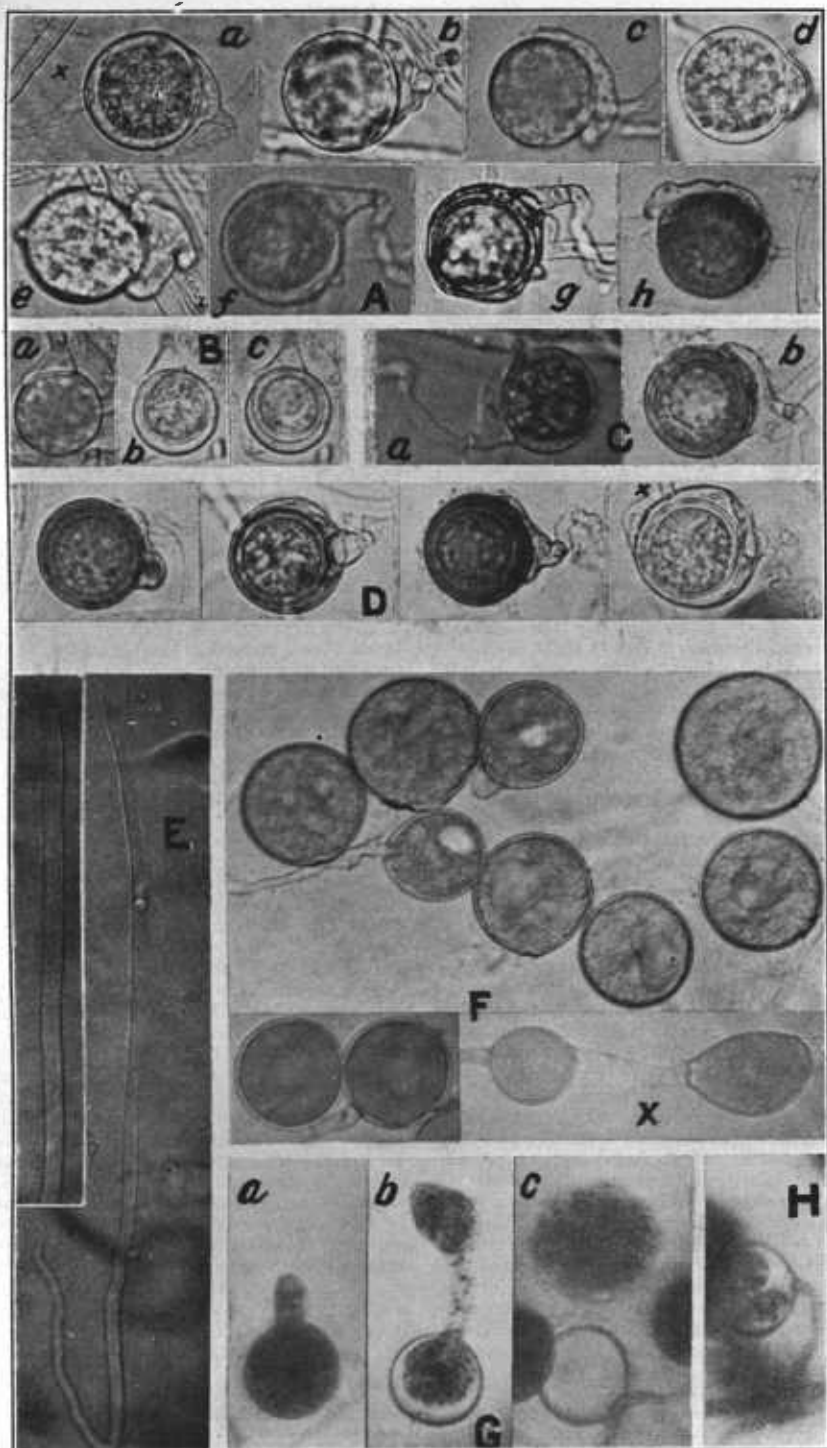
The tube may issue from any part of the surface. Its contents are at first clear and highly refractive; later on granules from the sporangium gradually wander in. During the next 10 to 15 minutes the tube continues a slow growth, which ceases when a length of a third to half the diameter of the sporangium has been reached. In the meantime the sporangial contents have been in slow motion, the vacuole and small dark spots changing their position gradually and irregularly. The tip of the tube suddenly blows out into an expanding bubblelike vesicle, whose wall is barely distinguishable from the surrounding medium; the sporangial contents ooze into it as it expands. The protoplasm within the sporangium breaks away from the wall opposite the tube, leaving lengthening fine strands attached to it. The vacuole, when present, does not appear to enlarge but is included in the general mass squeezing through the tube, and may break if too large.

After the greater part of the contents has entered the bladder, the passage of the remainder gives the impression of a pulling through by the force of surface tension tending to round up the entire protoplasmic mass into the vesicle. Infrequently, part of the contents breaks off and remains within the sporangial wall. Here it differentiates into a very few zoospores which fail to escape (Pl. 4, H) and finally degenerate.

The sporangial wall collapses slightly in places and becomes irregularly angular. The contents are now clear except for a few irregular strands which connected the wall and the viscous departing protoplasm. The latter does not completely fill the vesicle and is at first irregular in outline. It gradually assumes a smooth rounded form through the continued action of surface tension, and presents the same general appearance as when within the sporangium, except that considerably more space is occupied owing to imbibition of water in the absence of rigid confining walls. After a short period of quiescence, clear furrows and wedges appear on the periphery, from which vague lines of demarcation grow in toward the center of the mass. A rocking movement begins and increases in vigor as the lines become clearer and delimit definite re-

#### EXPLANATORY LEGEND FOR PLATE 3

- A.—Oospore and mycelium, in turgid cells. Stained. Oospore contracted, antheridium lobed  
 B.—Oospores in crushed cells. Stained. Antheridium appressed; oosphere immediately subjacent  
 C.—Section of pith. Note turgid infected cells, demarcation, crushed cells, and cavity  
 D.—Oospores in crushed and turgid (at *z*) pith cells



*Geranium* Stemrat

(For explanatory legend see p. 409)

gions of protoplasm. Cilia may be seen lashing about at the margin, which is now well indented. The general mass increases in volume with the appearance of single vacuoles within the zoospores, which are now well marked off and begin to separate at their tips. They move slightly apart and over each other for a short period, then break apart singly or in entangled pairs and swim away through the disrupted membrane. In one case the first few zoospores to escape left through the same part of the vesicle; but as a rule there is no localized point of exit, the vesicle wall apparently splitting at several places through the impacts of the moving zoospores. In some cases the entire mass of entangled zoospores may break out of the membrane and move off some distance before separation takes place.

No trace of the vesicle can be seen after the escape of the zoospores. The open tube, which has acquired enough wall material to insure rigidity, remains on the old sporangial wall. It is now rarely more than one-third the diameter of the spore, and the additional length observed in the early stage of germination evidently had gone to make up the vesicle.

The process of zoospores formation, from the protrusion of the tube to the escape of the zoospores, takes about 15 minutes. It is more or less synchronous in most of the sporangia in a drop, which may be seen swarming with zoospores half an hour after sowing.

**ZOOSPORES.**—From 10 to 26 are formed from each sporangium. They are broadly lenticular,  $5.9\ \mu$  to  $8.5\ \mu$  wide by  $10.6\ \mu$  to  $11.5\ \mu$  long, contain a single small round vacuole surrounded by finely granular protoplasm, and bear two cilia at the hilum (Pl. 5, A). After swimming around for half an hour to an hour, they come to rest, and the ciliary motion gradually ceases as the zoospore rounds up. A very slender germ tube is put forth, which broadens back to

the base as it grows in length and branches out into a mycelium.

Asexual fruiting bodies from older cultures show an increased tendency to direct germination, particularly in the case of those with large vacuoles. The tubes do not differ at first from those extruded in the indirect process, except that more than one may be formed from a single spore. The tips continue to grow, branch, and form a mycelium.

**OOSPORES.**—These are formed in great abundance within the cells of diseased tissues, on oatmeal agar, and geranium agar. They are globose, smooth walled, lie free within the oogonial wall, and average  $16.18\ \mu$  in diameter (300 measurements), ranging from  $11.32\ \mu$  to  $20.85\ \mu$ . The contents are hyaline, with fine granules and oil drops surrounding a large rounded excentric vacuole half the diameter of the oospore. The walls are hyaline when young, later becoming yellow to brown. The formation of discolored oxidation products on substrata is evidently a factor in wall coloration, since oospores from oatmeal and corn-meal agar, which are not discolored, remain light yellow, whereas oospores from host tissue and geranium agar, both of which are browned, are dark walled.

The oogonial wall is smooth or slightly collapsed, and often bears traces of the oogonial stalk.

The most characteristic feature of the mature sexual fruiting body is the antheridium. It is one-celled, persistent, and varies in shape from a trumpet form flaring out at the region of attachment, to a broad irregularly lobed mass clasping or wrapped around a large part of the oogonium and fused with it. The existence of a lateral pressure is indicated by the collapse of that part of the empty oogonial wall immediately underneath. The cylindrical or clavate type so characteristic of most *Pythium* spp. has not been observed in host tissue.

#### EXPLANATORY LEGEND FOR PLATE 4

- A.—Oospore formation, early stages (a to h). Note clasping antheridium; stalked oogonium (at ax)
- B.—Three stages in maturation of same oospore (a to c)
- C.—Oospores in contracted oosphere stage (a) and exospore stage (b)
- D.—Mature oospores
- E.—Hyphae, showing cylindrical nature and rounded tips
- F.—Sporangia-terminal, and intercalary (at x)
- G.—Sporangial germination: (a), Tube protruded; (b), contents partly in vesicle; (c), contents extruded, undifferentiated
- H.—Three zoospores entrapped in sporangial wall, after most of the undifferentiated contents had emerged

Observations on development and fertilization have been made repeatedly on living material growing in hanging drops of oatmeal decoction, in which the young fruiting bodies appear within two days (Pls. 4 and 5). The antheridium is in contact with the oogonium at a very early stage, before either has been cut off by a septum. At this time it is larger than the oogonium and partly wrapped around

it, and presents the characteristic flaring shape. It arises from a neighboring hypha, from an adjacent branch of the same hypha, or immediately beneath the oogonium. The latter is formed by the swelling of a hyphal tip, finally outgrows the antheridium, and both are cut off by septa (Pl. 5, B). Mature oogonia average  $18.56 \mu$  in diameter (300 measurements) and range from  $13.2 \mu$  to  $23.31 \mu$ .

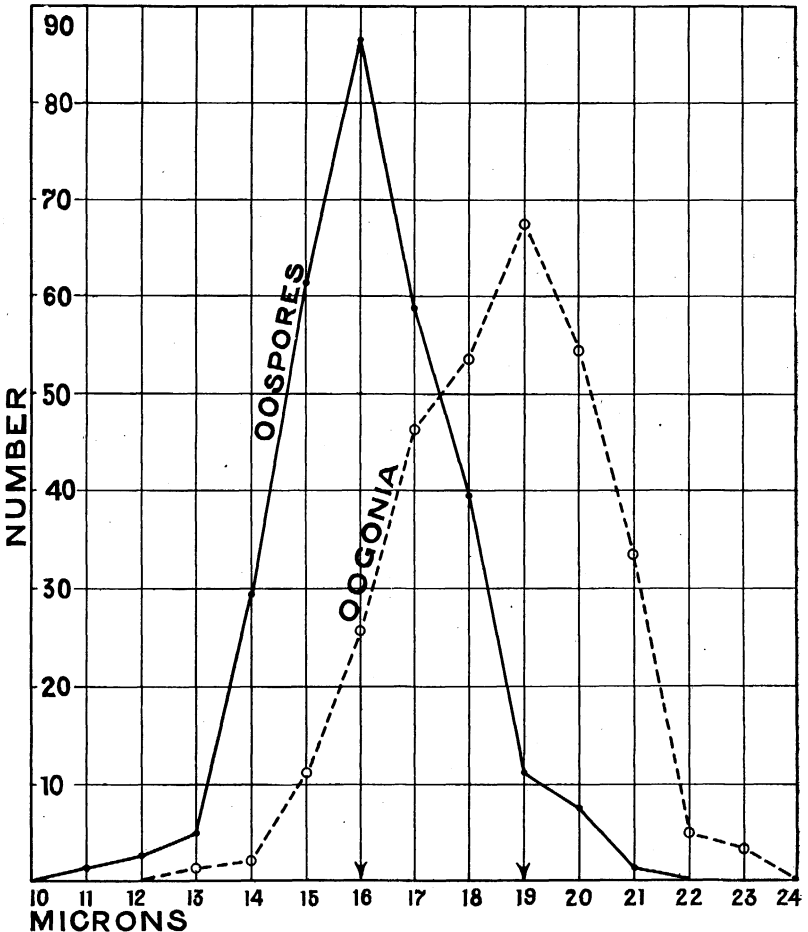
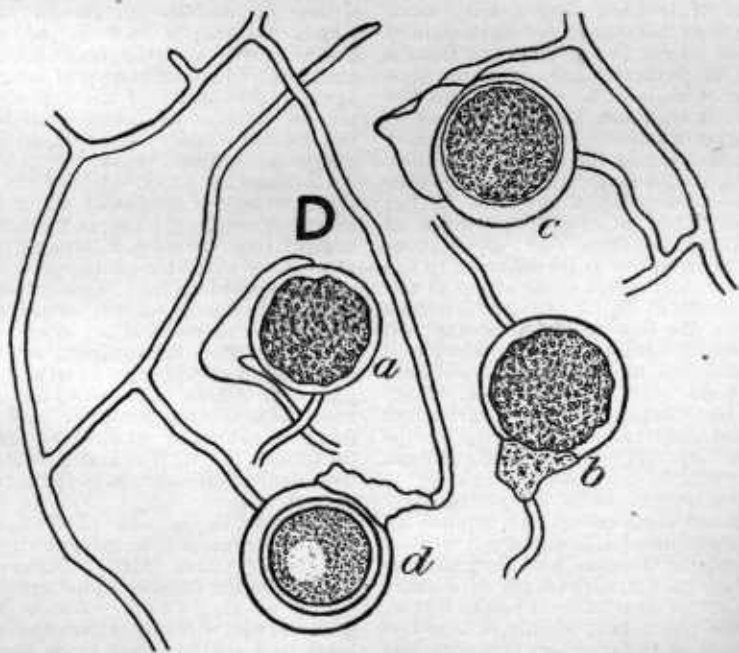
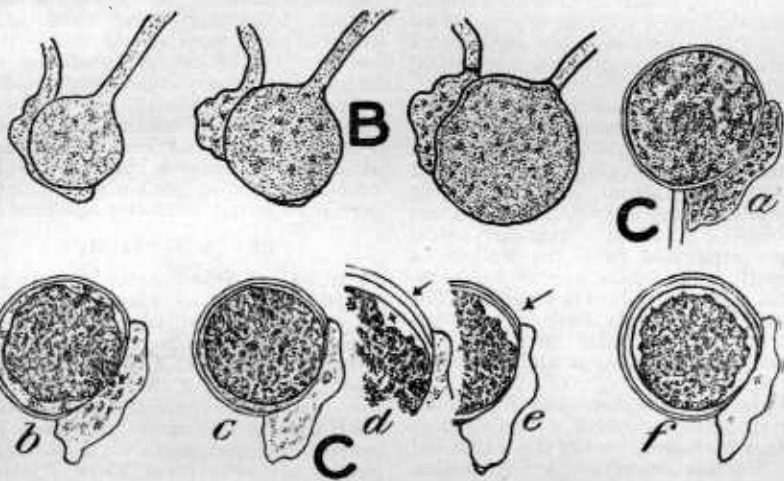
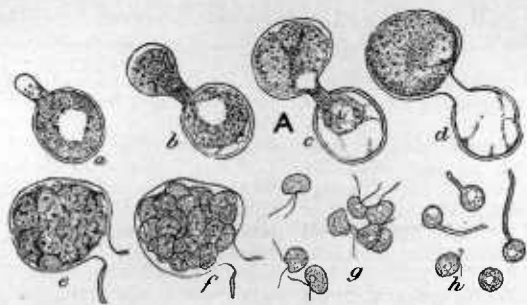


FIG. 2.—Frequency curves of oospore and oogonium measurements, 300 of each

#### EXPLANATORY LEGEND FOR PLATE 5

- A.—(a to h), stages in formation and germination of zoospores  
 B.—Three stages in early growth of sexual bodies  
 C.—Fertilization: (a), Beginning of oosphere contraction; (b), passage of antheridial contents into subjacent oosphere through hole in fused walls; (c), oosphere rounding up; (d, e, f) exospore wall formation by clear band extending around oosphere periphery. The clear band in (e) and (f) is drawn at twice actual width, to render it visible at scale of reproduction  
 D.—Oospores, showing attachment on slender stalk and relation to antheridium



Geranium Stemrot  
(For explanatory legend see p. 410)

The protoplasm in both antheridium and oogonium is nonvacuolate and at first finely granular. The oogonial contents gradually become denser and lumpy and undergo a slow irregular motion, accompanied by the appearance and disappearance of darker particles similar in shape and size to the nuclei observed in stained sections. The antheridial contents have also become coarser but remain much more hyaline. Considerable pressure is exerted by the closely appressed antheridium, evidenced by the depression of the oogonial wall and the consequent irregularity of the otherwise spherical oogonium.

Contraction of the oosphere is initiated by the appearance of irregular clear wedges at the periphery, first visible near the region of attachment to the antheridium and progressing around the periphery until the oosphere acquires a lumpy, irregularly oval shape, separated from the wall by a perfectly clear space except for occasional thin protoplasmic strands. The antheridial contents have also undergone a slow, irregular streaming and appear most hyaline at the base near the septum.

When the oosphere has fully contracted, it is invariably found in close contact with some part of the wall fused with the antheridium. A fertilization tube has not been observed, although dozens of fruiting bodies have been watched at this stage; nor have stained sections so far indicated more than a fusion of antheridium, oogonium, and a flow of antheridial content into the subjacent oosphere, through a break in the region of fusion. In living material a slow emptying of the antheridium may be seen at this stage. In the few cases where fertilization took place in the optical plane a cylindrical mass of protoplasm could be seen slowly progressing from the antheridium into the oosphere through a clear space in the fused wall (Pl. 5, C, b). An interface between the two masses of protoplasm was clearly visible, but was wavy and irregular, not smooth, like the fertilization tube of *P. de baryanum*, which was also kept under observation. The antheridium is left empty except for a few strands and a large oily globule at the mouth.

After fertilization the irregularly contracted oosphere quickly rounds up into a smooth ball, slightly increased in volume. Oospore wall formation is initiated by the appearance of a short very narrow clear strip (Pl. 5, C, d at x) at some point tangentially within the periphery of the oosphere; this extends slowly around the circumference until

the contents are inclosed by a narrow clear band, whose outer edge is perfectly smooth and circular, the inner edge being uneven and warty. (Pl. 5, C, d, e, f.) A contraction begins, similar to that of the original oosphere, except that a perfectly homogeneous refractive material is left in the wake of contraction. This proceeds until the full thickness of the wall is laid down, whereupon the irregular periphery of the contents smooths out into a circle. In the meantime the contents have become less lumpy and more finely granular; two dark round bodies may be seen in the center, probably the fusion nuclei; a vacuole appears and enlarges, and minute oily globules appear in the surrounding protoplasm. The antheridium shrinks and remains attached to the oogonial wall as the oospore attains maturity. Oospore germination has not been observed.

#### CULTURAL STUDIES

Growth of this fungus was studied on 16 media in four culture series, each of which included triplicate inoculations on all media. Cultures were kept in the dark at room temperature (18° to 25° C.) for three months. In the main the terminology suggested by Harsch and Long (11) has been followed. Comparisons over a four-year period with the other three *Pythium* spp. have shown a constancy of characters of definite diagnostic value, which has emphasized to the writer the necessity and the feasibility of a standardized procedure and terminology for the study of cultural characters of fungi, such as is available to the bacteriologist. Growth on these media is described below:

**CORN-MEAL AGAR.**—Upper half of slant covered with downy white aerial growth, becoming loose and cobwebby toward base of tube, flattening down after two weeks to form a hyaline, smooth, sodden mat; closely parallel hyphæ at edge of colony, resulting in a striking combed-silk effect when viewed by transmitted light; irregular radiating patches of varying density in older part of colony. Growth on corn-meal agar plates prostrate and submerged; otherwise similar to growth in tubes (Pl. 1, B). Differ markedly from other *Pythium* spp. in the combed-silk effect.

**CORN-MEAL FLASKS.**—Felted, compact white growth forming a dry mat about 2 or 3 mm. thick. Differs only in forming the more compact growth.

**OATMEAL AGAR.**—White aerial growth well defined after five days, loose and cottony, but more compact in central part of colony; after 14 days

becoming appressed and felted on upper half of slant, with more open texture toward base; drying down in three months to a uniformly compact white mat. No marked difference.

**CARROT AGAR.**—Growth prostrate, forming a thick gray sodden mat, wet-shining, irregularly wrinkled; aerial after three weeks, resulting in a uniform woolly white mass; later drying down to a prostrate deeply wrinkled dry mat bearing small floccose areas on upper part of slant, with loose cottony masses at base (Pl. 1, C.) Radiating irregular patches visible by transmitted light in central part of colony. Differs from other three species in absence of early aerial growth and in characteristic wrinkling.

**STRING BEAN AGAR.**—Prostrate dry mat deeply wrinkled in center; aerial growth after three weeks, forming a closely appressed felted mass, white at upper part of slant, becoming sepia brown toward center of slant, with loose cobwebby hyphæ at base; flattening down in three months to a thin dry mat, with marked wrinkling in center and the characteristic patchy appearance by transmitted light.

**POTATO AGAR.**—Differs from other three species in absence of early aerial growth and in wrinkling. Prostrate, smooth, sodden mat, bearing short downy hyphæ after 10 days, flattening down after three weeks to a gray, wet mat, slightly wrinkled at base of slant.

**POTATO DEXTROSE AGAR.**—Prostrate sodden mat, bearing tufts of loose to felty white hyphæ; deeply wrinkled in center after three weeks, light-grayish olive, with cobwebby tufts of aerial hyphæ scattered over lower half of slant; compact, felted tufts on upper half. Differs in presence of wrinkling from other three species.

**SUGAR BEET AGAR.**—Compact white aerial growth after one week, bearing olive-green slimy masses of oospores on center of slant; after two weeks, loose woolly-white hyphæ, partly overgrowing olive-green masses, later flattening down to a sodden, wrinkled, gray mat with scattered cobwebby hyphæ in lower part of tube. Characterized by presence of olive-green masses.

**CONGO RED BEEF PEPTONE AGAR.**—Very scanty surface growth, consisting of a few widely scattered prostrate hyphæ; surface of slant becoming dry and glossy; abundant submerged growth, filling the agar cylinder with long closely parallel hyphæ; very marked combed-silk effect and radiating patches by transmitted light; color of medium changed to deep Indian purple (15) within two weeks. No color change in others.

**BEEF INFUSION PEPTONE AGAR.**—Thin, sodden gray mat showing characteristic combed-silk effect and radiating areas; no aerial mycelium developed. Differs in having the combed-silk effect.

**GERANIUM DECOCTION AGAR.**—Growth similar to that on beef agar, except that the medium is discolored a greenish brown several millimeters in advance of growth. Growth much slower than that of other *Pythium* species.

**STERILIZED GERANIUM STEM** (with distilled water in tube).—Blackened in three days except part under water; scanty aerial growth on surface of stem; abundant diffuse growth out into the water. No marked difference.

**POTATO CYLINDERS.**—Thick, sodden gray mat, bearing a few small tufts of short downy hyphæ; slant covered after three weeks with compact downy to felty white mycelium, becoming loose and cottony at base of cylinder. No marked difference.

**SUGAR-BEET CYLINDERS.**—Felted white mass within two weeks, closely appressed, becoming cottony toward base; after two months forming a uniformly compact dry white mat. Differs in extreme compactness of growth from each of the others.

**STRING BEAN PODS** (with distilled water in tube).—Sodden gray mat covering pod, bearing scattered tufts of short downy hyphæ, which mat down after three weeks or persist in spots; thick gelatinous mass floating on the water in the tube, with many hyphæ diffusing underneath. No marked difference.

**SOYKA RICE.**—Abundant white cottony growth, becoming felty and compact after three weeks; no color change after three months. Differs in the more profuse growth from the three others.

#### VIABILITY

Duplicate tube cultures on various media, which had been kept at room temperatures (18° C. in winter to 29° in summer) for 6 to 16 months, were tested for viability in two experiments. A sterile decoction of Quaker oats was poured into each tube and removed after absorption of fluid by the dried agar. Melted corn-meal agar, cooled down to 38°, was then poured on the slants; the tubes were replaced in the incubator and examined up to three weeks. Transfers from cultures which showed growth on the fresh agar were made to oatmeal agar and carrot agar for identification and comparison with similar transfers from a stock culture. Results are given in Table I.

TABLE I.—Viability experiments

Medium	Date in-oculated	Date tested	Time elapsed	Observations (duplicate tubes)
Oatmeal agar.....	Dec. 31, 1919	Aug. 25, 1920	7 months, 25 days.	Alive in one tube.
Potato agar.....	Jan. 26, 1920	.....do.....	7 months.....	Alive in both tubes.
Do.....	Feb. 25, 1920	.....do.....	6 months.....	Do.
Oatmeal agar.....	Sept. 8, 1921	Aug. 22, 1921	11 months, 14 days.	Dead in both tubes.
Corn-meal agar.....	.....do.....	.....do.....	.....do.....	Alive in one tube.
Congo Red agar.....	.....do.....	.....do.....	.....do.....	Dead in both tubes.
Beef agar.....	.....do.....	.....do.....	.....do.....	Do.
Potato agar.....	.....do.....	.....do.....	.....do.....	Do.
Potato dextrose agar.....	.....do.....	.....do.....	.....do.....	Do.
Carrot agar.....	.....do.....	.....do.....	.....do.....	Alive in both tubes.
Bean agar.....	.....do.....	.....do.....	.....do.....	Alive in one tube.
Steamed rice.....	Apr. 14, 1920	.....do.....	16 months, 8 days..	Dead in both tubes.
Sterilized geranium stem.....	Apr. 17, 1920	.....do.....	16 months, 5 days..	Do.

The experiments indicate viability after eleven and a half months at room temperature on carrot agar, bean agar, and corn-meal agar; and after seven and nearly eight months on potato

agar and oatmeal agar, respectively. The fungus was not recovered after 11 to 16 months on seven other media used. Viability appears to be connected largely with the water-retaining

power of the media. Death occurred much sooner on plate cultures, where the large surface and thin layer of medium leads to rapid desiccation. On October 8, 1923, sterile tap water was poured over corn-meal agar plate cultures 3 and 6 months old and bone dry. Absorption and softening soon took place, but no germination of the numerous oospores and sporangia was observed after three days, nor did growth occur on replacing the water with fresh cornmeal agar. Stock cultures in oatmeal agar tubes have, however, been kept in the icebox for 18 months without loss of viability.

TEMPERATURE RELATIONS

The Figure 3 outlines the growth curves averaged from two experiments with plates in ice thermostats and warm incubators, ranging from 2° to 37.5° C. Triplicate plates of corn-meal agar were placed in each compartment and measurements of the colony diameter were made at 24-hour intervals. Inoculations were made from a 3-day-old corn-meal agar plate, which was cut up into one-sixteenth inch

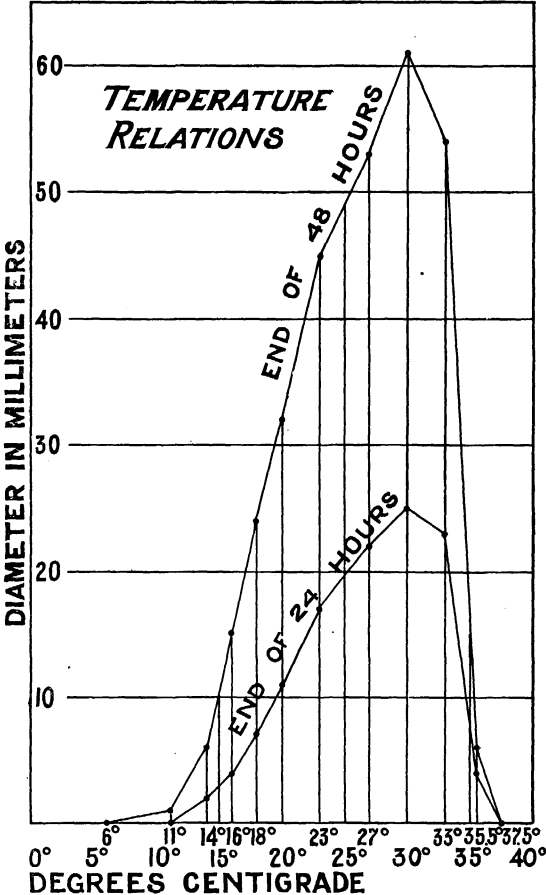


FIG. 3.—Graphs showing colony diameters at 2°- 37.5° C. for 24 and 48 hours



squares, each square then being planted in the center of the fresh plate. Before inoculation all plates were kept overnight in their respective compartments to avoid lag effects.

No growth takes place at 11° or at 37.5° within 48 hours or for any subsequent period at the latter temperature. Very slow growth is evident at 5° after 144 hours (3 mm., not shown in fig. 3). The optimum temperature centers around 30°. A marked difference in temperature ranges above and below the optimum may be observed, the growth rate falling away much more rapidly above the optimum temperature.

## GENERAL DISCUSSION

### TAXONOMY

The general characters of this fungus and the fact that the zoospores are not formed within the original sporangial wall, but within an evanescent vesicle containing the undifferentiated extruded contents of the sporangium, place this organism in the genus *Pythium* (Pringsheim). Preliminary complete differentiation before entrance into the vesicle has not been observed, although scores of sporangia have been germinated and studied; the only other method of germination noted was by germ tube, as described above for older sporangia.

The characters of the sporangium and oospore place this organism in Butler's subgenus *Sphaerosporangium* (6), among the species with smooth-walled oospores lying free in the smooth oogonium (*P. de baryanum*, *P. ultimum*, and *P. vexans*). It differs from the first, which was studied at the same time, in: (a) Smaller oospores and oogonia; (b) finer mycelium; (c) very marked cultural differences; (d) inability to attack water cress, cucumbers, and radishes; (e) preponderance of zoosporangia rather than conidia; (f) the very characteristic antheridium. It differs from *P. ultimum* in being parasitic, in producing zoosporangia rather than conidia (which is a distinguishing character of *P. ultimum*), and in the shape of the antheridium.

In this last character it comes nearest to De Bary's *Pythium vexans*, which he characterized (1, 2) by: (a) The peculiar insertion of the oogonium, which is sessile on the outside of the mycelium or inserted with a broad base into the mycelial tube; (b) the broad appressed antheridium fused to the oogonium, although his figure (2, Pl. 5, fig. 3) shows one clavate antheridium; (c) saprophytic habit; (d) no sporangia or conidia were observed. But-

ler (6) adds that the tapering of the hyphae into very fine filaments distinguishes it from any other species he studied. He was more successful than De Bary in finding sporangia, which were "rare, scarcely ever spherical or oval, but irregularly pear-shaped, ovoid or subangular."

The organism described in this paper resembles *P. vexans* in the shape of the broad appressed antheridium, but differs in the following respects: (a) The oogonia are not inserted with a broad base, but are borne on a slender stalk (Pl. 5, B, C, D); (b) it is parasitic on *Pelargonium* and *Coleus*; (c) sporangia are formed abundantly on various media and are oval to spherical (Pl. 4, F), except when intercalary; (d) the hyphae are cylindrical with rounded tips (Pl. 4, E) and do not taper to fine points. It is considered a distinct species, for which the name *Pythium complectens* n. sp. (referring to the clasping antheridium) is proposed. The technical description follows.

***Pythium complectens*, n. sp.**  
Hyphae coenocytic, hyaline, 1.70  $\mu$  to 4.85  $\mu$ ; cylindrical with rounded tips, forming a strongly parallel silky growth on solid media; acid produced changing color of Congo Red agar; sporangia abundant, vacuolate, produced singly, spherical when terminal, oval to sub-spherical when intercalary, without papilla, average 21.8  $\mu$  in diameter, range 16.4  $\mu$  to 27.3  $\mu$ , germinating by extrusion of undifferentiated contents into vesicle in which zoospores are formed, not proliferating; zoospores broadly lenticular, with two cilia at hilum, with single vacuole, 10 to 26 formed per sporangium, 5.9  $\mu$  to 8.5  $\mu$  wide by 10.6  $\mu$  to 11.5  $\mu$  long, rounding up and germinating by a tube; oospores single, smooth walled, spherical, free in the oogonium, wall light yellow to sepia brown, abundant in host tissues, average 16.2  $\mu$  in diameter, range 11.3  $\mu$  to 20.8  $\mu$ ; oogonia smooth, sub-spherical, borne on a slender stalk, average 18.6  $\mu$  in diameter, range 13.2  $\mu$  to 23.3  $\mu$ ; antheridium single, one-celled, arising from adjacent hypha or below oogonial stalk, persistent, varying from a trumpet shape flaring out at region of attachment, to a broad irregularly lobed mass clasping a large part of the oogonium and fused with it. Parasitic on *Coleus* and *Pelargonium* cuttings, causing a black stem-rot; inducing a resistance reaction in the latter host characterized by the formation of a cork cambium, barring further progress of the hyphae after infection has proceeded some distance from the point of inoculation.

## FERTILIZATION AND MATURATION PHENOMENA

A striking feature of fertilization is the absence of a fertilization tube, coincident with the close contact of the contracted oosphere with some part of the oogonial wall immediately underneath the clasping antheridium. Butler (6, p. 52) found no fertilization tube in a species of *Aphanomyces*, in which the antheridium closely encircled the oogonium, but thought it possible that a nucleus was transferred before the oosphere receded from the oogonial wall. He figures a fertilization tube, however, in *P. vexans*, which has the same encircling type of antheridium, but no close contact of the fused wall and oosphere, so far as can be judged from his drawings. The close relation of oosphere, oogonial wall, and antheridium in the species here described is brought out clearly in photographs of living material just prior to or at the time of fertilization. (Pl. 4, Aa, Af, Ba, Bb, Ca.) Under these conditions the direct passage of the antheridial contents into the oosphere through a hole dissolved in the walls between is thoroughly compatible with the clasping nature of the antheridium, which insures sufficient adhesion to eliminate the necessity of the anchorage afforded by a fertilization tube in species having a narrow clavate antheridium. The fact that a lateral pressure is exerted by the antheridium is well shown by the depressed condition of the oogonial wall below it, displacing the otherwise spherical shape of the oogonium (Pls. 4, 5, all oospore figures).

The inception of the exospore wall immediately upon fertilization and rounding up of the oosphere has not been described in detail in this genus so far as the writer is aware; most authors record the appearance of the complete membrane without figuring possible intermediate stages. Trow (22) states that "the egg rounds itself off and appears covered with a membrane before the last traces of protoplasm leaves the antheridium." Miyake, working with *P. de baryanum* (13), uses similar terms: "Soon after the discharge of the antheridial contents into the oosphere a thin membrane is formed around the latter. This is the beginning of the exospore." The figures of both authors show a completely encircling membrane of appreciable thickness, immediately following the highly contracted, smooth-outlined oosphere. De Bary noted a thin hyaline membrane around the oosphere before fertilization in *P. de baryanum*; its first figured appearance is a line

almost completely encircling the still rough oosphere (3, Pl. 1, fig. 3, 4). Ward (23), working with the same species, states that "meanwhile [during fertilization] a very delicate skin had been formed over the now smooth exterior of the oosphere," but his figures do not show how it was formed. The general impression left by these authors is of a simultaneous appearance of a thin hyaline membrane over the entire oosphere. This was not found to be the case in the species here studied, in which the membrane is first visible just within the smoothed periphery as a short, narrow hyaline strip (really a disk in terms of three dimensions) which gradually extends tangentially around the periphery until the entire oosphere is clothed with a narrow, hyaline membrane (Pl. 5, C, d, e, f).

## SIGNIFICANCE OF THE PYTHIUM TYPE OF SPORANGIAL GERMINATION

The normal process of sporangial germination in this genus results in the transformation of the entire sporangial contents into zoospores and their facile escape through the very delicate vesicle wall without any waste of zoospore-forming material or inherent obstacles to dissemination when once formed. It was frequently observed, however, that the undifferentiated mass within the vesicle sometimes moved off (as if the bladder had been disrupted prematurely), failed to differentiate, and degenerated into a mass of slime and globules. This was frequently correlated with impacts by freshly formed zoospores, singly or in groups, from near-by sporangia, and must unquestionably occur in nature through mechanical injury by the abundant motile microscopic fauna and flora of the soil. Another abnormal condition, observed in only two cases, was the disjunction of the protoplasm during its passage through the tube; most of it entered the vesicle and underwent the usual process, while the part broken off and left behind in the sporangium failed to emerge, but formed a few normal zoospores which remained entrapped and finally degenerated (Pl. 4, H). The rarity of this condition in *Pythium* is indicated indirectly in that incomplete exit of the sporangial contents, resulting in entrapped zoospores, is neither mentioned nor figured in Butler's monograph (6) nor in Ward's detailed accounts (23).

Instances of imprisoned zoospores are, however, not uncommon in allied genera in which they are completely

differentiated before emergence. Rosenbaum, working with *Phytophthora* (16), states that "frequently for some reason a few of the swarmspores do not emerge with the majority." In forms in which the vesicle occurs (accompanied by preliminary differentiation in the original sporangium), he finds that "after the liberation of the swarmspores, the vesicles begin to contract, all signs of the opening disappear, and if any zoospores remain they are unable to escape." Coker, in his recent monograph of the *Saprolegniaceae* (7), notes that "in both *Saprolegnia* and *Achlya* it frequently happens that the discharge of the spores is only partial, a few, or even a good many spores being left in the sporangium. These retained spores may emerge from their cysts as normally, for a second swimming stage, moving about within the sporangium until they find their way out by its mouth, if they ever do."

Correlation of these observations indicates that the *Pythium* type of zoospore formation *per se* is by far the better mechanism for securing maximum dissemination with minimum loss of spore-forming material for the following reasons:

(1) The migrating protoplasmic mass, covered as it is by a single plasma membrane, is kept together by cohesion and surface tension, both of which are powerful enough (except in rare and clearly pathological cases) to force the entire mass out as a whole, once emergence has been initiated by the propulsive force engendered by imbibition and swelling of a colloidal aggregate in the presence of an available opening. The operation of these factors is clearly evident to anyone watching the pulling together of the migrating mass when half way through the tube, and its subsequent rounding up when wholly within the elastic vesicle. Compared with other genera, it is in sharp contrast with the emergence singly of a collection of fully formed zoospores, the vanguard of which must be subjected to the propulsive force of swelling, which is necessarily exhausted when the total potential swelling of the remaining individuals is equal to the volume of the sporangium; the exit of the remainder, as De Bary (4), Coker (7), and others have shown, is largely dependent on their connection with the preceding spores by delicate protoplasmic threads and ciliary entanglements rather than by their own unaided efforts. Considering the fragility of these connections and the miniature turmoil at the time of emergence, it is not surprising that zoospores are often left behind

and fail to escape, as the above-quoted authors have observed. In the *Pythium* type of egress, however, the entire sporangial contents are removed *en masse* and under one enclosing membrane, thereby incurring a minimum risk of wasting spore material by imprisonment in the sporangial walls.

(2) Provision for maximum facility of dissemination, once the zoospores are formed, is secured in *Pythium* by the presence of an extremely tenuous, fragile membrane around the zoospore mass, capable of disruption with the greatest ease and thereby enabling all zoospores formed to swim away, as contrasted with the rigid walls surrounding the zoospores in other genera.

These conditions and their results—emergence *en masse* and fragility of the vesicle membrane—point clearly to the above conclusion as to the greater intrinsic efficiency of the *Pythium* type of sporangial germination, and to the interpretation that this type constitutes a definite adaptation securing minimum waste of spore-forming material combined with maximum ease of dissemination. That it did not become the prevailing type in zoospore-forming fungi can be traced to another factor, the clue to which is afforded in the above-recorded observation that the delicate vesicle containing the undifferentiated mass was susceptible to impacts leading to mechanical injury and degeneration before zoospores could be formed. It is clear that maximum dissemination is here obtained at the expense of protection at a critical stage, such as is afforded in genera in which the zoospores are formed within the rigid sporangium. The great advantage of the latter method lies in the fact that some at least of the swarmspores are sure to escape and perpetuate the race; in the *Pythium* type, the whole output of the sporangium is lost if the undifferentiated mass is mechanically injured in its exposed position. Evolutionary tendencies in matters of reproduction in higher plants and animals are in the direction of specialized and well-protected off-spring in small numbers rather than along the lines of quantity dissemination of the unprotected many; it is only necessary to consider this to see that a specialized adaptation which secures the latter end, such as we have in *Pythium*—admirable a mechanism as it intrinsically is—yet insufficiently proof against unfavorable conditions, can not imprint itself permanently on derived genera. Hence, in the closely allied and probably derived *Phy-*

trophthora, the tendency to the well-protected method of differentiation within the original sporangium finds full expression; the vesicle present in some species no longer serves to hold the undifferentiated spore mass but remains as a functionless inheritance from *Pythium* like ancestors, finally disappearing in other members of the genus.

#### RESISTANCE PHENOMENA

The formation of a protective cork cambium tending to inhibit the progress of infection, such as occurs in this disease, has been noted elsewhere by various workers. Lutman (12) interpreted potato scab as a successive series of cork layers laid down in advance of the parasite, which, however, was apparently powerful enough to pass through each layer and stimulate the production of another layer deeper in the tissues. He noted the disappearance of starch from healthy cells in the vicinity of the cork cambium, a condition which is also present in this geranium disease. Dufrenoy (8) reports protective cork formation in a chestnut disease. Tisdale, working with flax wilt (21), noted suberization of groups of cells adjacent to those attacked by *Fusarium lini*, and pointed out the necessarily chemical nature of the initial interacting forces involved. Protective cork formation accompanied by disappearance of starch from adjacent healthy cells is figured by Erwin F. Smith in a potato tuber rot caused by *Bacillus caratovorvus* (19, fig. 174).

The effectiveness of suberized cell walls in preventing the progress of infection has been emphasized by Shapovalov and Edson (17) in their work on wound cork formation. In the case of *Pythium* it is in accord with the mechanical nature of hyphal penetration as interpreted by Hawkins and Harvey (9), although the high resistance of suberized walls to solution by a large number of reagents should not be overlooked. The inability to obtain infection on uninjured geranium stems with the organism here reported, and the low amount of infection on cuttings made two days before inoculation, are clearly correlated with difficulty in penetrating cork layers, which, as related to the former case, are often 10 cells thick beneath the cuticularized epidermis.

It is of interest to note that the organism at present under consideration produces a diffusible substance, evidently an acid, capable of changing the color of Congo Red agar to a deep

India purple (*vide* Cultural studies), whereas the other three *Pythium* spp. studied at the same time (to be reported on in a later paper) caused no color change in this medium; nor did the latter ever induce cork formation in inoculated plants, which usually succumbed completely. While the nature of the diffusible substance produced by the cork-inducing *Pythium* has not been determined, a possible cause and effect relation is clearly indicated in the correlation between its production and the presence or absence of a resistance reaction evidenced by the stimulation of cell division to form a cork cambium. Stimulation of cell division by diffusible substances produced by a parasite is considered by Kunkel (10) as a possibility in the case of the *Plasmodiophora* disease of cabbage; further evidence is available in the numerous experiments of Erwin F. Smith with *Bact. mori*, *Bact. solanacearum* (19) and particularly with known by-products of *Bact. tumefaciens* (18, 20).

#### SUMMARY

1. A stemrot of geranium (*Pelargonium*) cuttings caused by *Pythium complexens* n. sp. is here described.

2. The disease consists of a progressive basal blackening accompanied by pectinization and soft rot of pith and cortex. Infection stops at a sharp line of demarcation 20 to 40 mm. from the base within six to eight days after inoculation.

3. Stoppage of infection is due to a host resistance reaction manifested by the formation of a cork cambium completely across and within the stem, barring further progress of the hyphae after infection has already proceeded some distance from the point of inoculation. It is accompanied by the disappearance of starch from healthy cells in the vicinity of the cambium.

4. This reaction is specific for this particular host and organism, and was not found in the case of three other *Pythium* spp. studied which caused complete destruction, nor in the case of *Coleus* cuttings infected and completely rotted by this organism.

5. Characteristic signs of the disease can be caused with pure culture inoculations of this organism on any wounded part of the stem. Reisolation, reinoculation, and constant presence of characteristic oospores in undecayed parts of diseased tissues have established pathogenicity. *Coleus* cuttings are susceptible, but not cucumber, radish, or cress seedlings.

6. The hyphae of this fungus are hyaline, coenocytic, and cylindrical

with rounded tips. Sporangia are abundant in culture media, and are regularly oval to spherical. Germination is by extrusion of the undifferentiated contents through a short tube into an evanescent vesicle in which the zoospores are differentiated; germination by tube takes place in older sporangia. Proliferation has not been observed.

7. The oospores are smooth walled and lie free within the smooth oogonia. The latter are borne at the tips of slender branches. The antheridia are particularly characteristic, varying from a trumpet form to a broad, irregularly lobed mass clasping and fused with a large part of the oogonial surface.

8. Fertilization takes place by direct passage of the antheridial contents through a hole in the fused oogonial and antheridial walls into the contracted and immediately subjacent oosphere. A fertilization tube has not been observed.

9. Formation of the exospore wall is initiated by the peripheral spread of a narrow, clear band (a disk in 3 dimensions) within and around the smooth outlined oosphere, completely enclosing it.

10. Viability and cultural characters on 16 media are given in detail.

11. Optimum growth takes place at 30° C. The maximum temperature is 35.5°; the minimum 5° (144 hours).

12. Comparison of the *Pythium* type of zoospore formation with that in other genera points (a) to the greater intrinsic efficiency of the former type in view of the advantages herein discussed resulting from emergence of undifferentiated contents *en masse*, and the fragility of the vesicle membrane; (b) to an interpretation of this type as an adaptation which secures minimum waste of spore-forming material combined with maximum ease of dissemination, at the expense, however, of protection at a critical stage. That it did not become fixed in derived genera is an expression of the general evolutionary tendency toward protection of offspring rather than facility in dissemination.

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